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Abstract: Polyploidization is pervasive in plants, but little is known about the niche divergence of wild allopolyploids (species that harbor polyploid genomes originating from different diploid species) relative to their diploid progenitor species and the gene expression patterns that may underlie such ecological divergence. We conducted a fine-scale empirical study on habitat and gene expression of an allopolyploid and its diploid progenitors. We quantified soil properties and light availability of habitats of an allotetraploid Cardamine flexuosa and its diploid progenitors Cardamine amara and Cardamine hirsuta in two seasons. We analyzed expression patterns of genes and homeologs (homeologous gene copies in allopolyploids) using RNA sequencing. We detected niche divergence between the allopolyploid and its diploid progenitors along water availability gradient at a fine scale: the diploids in opposite extremes and the allopolyploid in a broader range between diploids, with limited overlap with diploids at both ends. Most of the genes whose homeolog expression ratio changed among habitats in C. flexuosa varied spatially and temporally. These findings provide empirical evidence for niche divergence between an allopolyploid and its diploid progenitor species at a fine scale and suggest that divergent expression patterns of homeologs in an allopolyploid may underlie its persistence in diverse habitats.

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Research

Fine-scale empirical data on niche divergence and homeolog expression patterns in an allopolyploid and its diploid progenitor species

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Summary

- Polyploidization is pervasive in plants, but little is known about the niche divergence of wild allopolyploids (species that harbor polyploid genomes originating from different diploid species) relative to their diploid progenitor species and the gene expression patterns that may underlie such ecological divergence. We conducted a fine-scale empirical study on habitat and gene expression of an allopolyploid and its diploid progenitors.
- We quantified soil properties and light availability of habitats of an allotetraploid *Cardamine flexuosa* and its diploid progenitors *Cardamine amara* and *Cardamine hirsuta* in two seasons. We analyzed expression patterns of genes and homeologs (homeologous gene copies in allopolyploids) using RNA sequencing.
- We detected niche divergence between the allopolyploid and its diploid progenitors along water availability gradient at a fine scale: the diploids in opposite extremes and the allopolyploid in a broader range between diploids, with limited overlap with diploids at both ends. Most of the genes whose homeolog expression ratio changed among habitats in *C. flexuosa* varied spatially and temporally.
- These findings provide empirical evidence for niche divergence between an allopolyploid and its diploid progenitor species at a fine scale and suggest that divergent expression patterns of homeologs in an allopolyploid may underlie its persistence in diverse habitats.

Introduction

Polyploidization is pervasive in plants (Otto & Whitton, 2000) and is one of the major mechanisms of speciation (Ramsey & Schemske, 1998; Soltis & Soltis, 2009). In allopolyploids (species with multiple genome sets from different diploid species), differential expression of homeologs (potentially redundant copies of genes derived from the progenitor species) has been speculated to reflect selective use of the homeologs in responding to various environmental conditions (Madlung, 2013; Yoo *et al.*, 2013), in addition to higher evolvability due to redundancy (Douglas *et al.*, 2015; Paape *et al.*, 2018). This, in turn, might enable allopolyploids to thrive in a range of environments. Consistent with this idea, manipulative experiments in laboratories demonstrated homeolog expression ratio changes in response to environmental factors such as temperature, water availability, and metal concentration (Liu & Adams, 2007; Dong & Adams, 2011; Akama *et al.*, 2014; Liu *et al.*, 2015; Paape *et al.*, 2016; but see Combes *et al.*, 2013). Recent empirical studies show that a small proportion (*c.* 1%) of all genes exhibits a ratio change, many of which are known to be involved in response to the specific conditions that were manipulated in the experiment (Akama *et al.*, 2014; Paape *et al.*, 2016). These results suggest that allopolyploids selectively express the homeologs relevant for an appropriate response to the environment. Although the expression patterns in the laboratory and in naturally fluctuating environments, or *in*

natura, may be distinct (Shimizu *et al.*, 2011; Yamasaki *et al.*, 2017; Song *et al.*, 2018), little is known about environment-dependent changes in the homeolog expression ratio of allopolyploids in natural habitats and about the divergence of expression patterns from progenitors.

Ecological differentiation enables allopolyploids to establish and persist amid well-established and possibly locally adapted diploid progenitor species, otherwise they would be excluded by competition (Hardin, 1960). Newly emerged polyploids may be prepared to exploit novel habitats, as they inherited divergent genomes of the diploid progenitor species (Levin, 2003; Paape *et al.*, 2020). This leads to an expectation that allopolyploids and their progenitor species occupy different habitats (niche divergence). Niche divergence between an allopolyploid and its progenitor species has been extensively studied using ecological niche modeling, where species geographic distributions are modeled in relation to large-scale climatic and topographic variables (Wake *et al.*, 2009). There is support for niche divergence, with intermediate and broad niche detected for allopolyploids, however, at the same time, several studies also report niche overlap between allopolyploids and their progenitors (Soltis *et al.*, 2010; McIntyre, 2012; te Beest *et al.*, 2012; Glennon *et al.*, 2014; D. E. Soltis *et al.*, 2014; P. S. Soltis *et al.*, 2014; Marchant *et al.*, 2016; Huynh *et al.*, 2020; López–Alvarez *et al.*, 2020). A possible explanation for niche overlap is the spatial scale of a study. Ecological niche modeling approaches typically evaluate environmental factors at a resolution of > 1 km^2. Multiple environmental factors are known to vary at fine spatial scale, including water availability, light availability, and soil properties (Denney *et al.*, 2020). These factors have been associated with niche divergence (Silvertown, 2004) and not yet incorporated in large-scale climatic modeling (www.worldclim.org). Under this circumstance, fine-scale empirical studies would have a better ability to detect niche divergence at fine scale. Furthermore, water availability varies not only spatially but also temporally (Denney *et al.*, 2020). Data from multiple time points should enable the evaluation of temporal variation in habitats as well as differential gene expression (Aubin-Horth & Renn, 2009; Aikawa *et al.*, 2010; Richards *et al.*, 2012; Alvarez *et al.*, 2015).

The genus *Cardamine* of Brassicaceae has long been a model to study ecological polyploid speciation (Howard, 1948; Hussein, 1948; Shimizu-Inatsugi *et al.*, 2017; Akiyama *et al.*, 2020; Sun *et al.*, 2020). The allotetraploid *Cardamine flexuosa* in Europe derived from diploid progenitor species *Cardamine amara* (genome size 217–273 Mb) and *Cardamine hirsuta* (genome size 198–225 Mb) (Johnston *et al.*, 2005; Mandáková *et al.*, 2013; Mandáková *et al.*, 2014; Gan *et al.*, 2016; in-house measurement) offers a promising system with which to study the niche divergence and associated homeolog expression of allopolyploids in comparison with the diploid progenitor species. *Cardamine flexuosa* is estimated to have emerged between 10 000 ka and 1 Ma (Mandáková *et al.*, 2014). The three species are common in central Europe (Carlsen *et al.*, 2009), and their distributions largely overlap at the scale of 5 km^2 in Switzerland (Supporting Information Fig. S1; Lauber *et al.*, 2012). However, anecdotal reports suggest niche divergence among species, as *C. hirsuta* plants are typically observed on roadsides and in ditches, *C. amara* on river banks and in wet woodlands, and *C. flexuosa* along forest roads (Urbanska & Landolt, 1978; Koch *et al.*, 2003; Lihová *et al.*, 2006; Grime *et al.*, 2007; Lauber *et al.*, 2012; Tedder *et al.*, 2015). In a manipulative laboratory experiment where the three species underwent drought, submergence, or fluctuation of the two, *C. hirsuta* performed the best in drought and worst in submergence, the opposite was the case for *C. amara*, and *C. flexuosa* performed similarly well in all treatments (Shimizu-Inatsugi *et al.*, 2017). The three species thus appear to show niche divergence along hydrological gradients, characterized by two distinct stresses – water-logging and drought – at the two ends of the gradient at a scale smaller than 5 km^2. Microarray analyses from the laboratory experiment showed that the gene expression induction pattern of *C. flexuosa* was similar to that of *C. hirsuta* under drought and to that of *C. amara* under submergence (Shimizu-Inatsugi *et al.*, 2017). It is yet to be shown how the gene expression pattern of *C. flexuosa* compares to that of the diploid progenitor species in field. Additionally, a major limitation of the usage of the *Arabidopsis* microarray was that it allowed the quantification of only the sum of the expression levels of two homeologs of only *c.* 46% of the entire genes. Recently, HomeoRoq, a tool for analyzing the homeolog expression ratio change in allopolyploids (Akama *et al.*, 2014; Kuo *et al.*, 2020), and the genome sequence of *C. hirsuta* (Gan *et al.*, 2016) became available, enabling evaluation of the expression of homeologs across genomes of *C. flexuosa*. These technical advances and findings from the laboratory experiment make *C. flexuosa* an allopolyploid with distinct ecologies and genomic tools. Such a study system is still rate (Soltis *et al.*, 2016), and thus *C. flexuosa* offers a unique opportunity for studying ecological transcriptomics of an allopolyploid and its diploid progenitors. However, quantitative data from natural habitats are still lacking.

Here, we conducted a fine-scale empirical study over two seasons to quantify water availability, soil properties, and light availability in the habitats of *C. flexuosa*, *C. amara* and *C. hirsuta* within their native range in Switzerland, in three areas in and around Zurich, and to analyze homeolog expression patterns of *C. flexuosa* in comparison with diploid progenitor. Based on previous studies, we hypothesized that the three species occur in different habitat; that is, the species differ in realized niche (Hutchinson, 1957). We expect that *C. flexuosa* would inhabit a wide water-availability gradient, including sites with fluctuating water availability, and that it would differentially express homeologs similarly to either of the diploid progenitor species depending on environments. We addressed the following questions: (1) What is the relative contribution of different environmental factors to the occurrence of the allopolyploid *C. flexuosa* as well as the two diploid progenitor species? What are the key environmental factors? (2) Along the gradients of environmental factors contributing to the occurrence of the species, is *C. flexuosa* in a distinct and/or broader range than *C. amara* and *C. hirsuta*? (3) How does the gene expression of *C. flexuosa* relate to that of the coexisting diploid progenitor species? (4) Is the gene (homeolog) expression pattern of *C. flexuosa* associated with environmental